

CLAIMS

1-17 (Canceled)

18. (Currently amended) A method for obtaining genetically modified human pluripotent hematopoietic stem cells, comprising:

a) contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of human pluripotent hematopoietic stem cells cultured with fibronectin and in the presence of an effective amount of a mpl ligand and a flt3 ligand, each ligand provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL, wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors, ~~and wherein said human pluripotent hematopoietic stem cells are CD34⁺Thy-1⁺Lin⁻ cells and can differentiate into any hematopoietic cell type, and wherein said concentration range does not cause differentiation of the human pluripotent hematopoietic stem cells;~~ and

b) obtaining said modified human pluripotent hematopoietic stem cells.

19. (Previously presented) The method according to claim 18, further comprising culturing the population of human pluripotent hematopoietic stem cells in the presence of a c-kit ligand in a concentration of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said vector.

20. (Currently amended) The method according to claim 19, further comprising culturing the population of human pluripotent hematopoietic stem cells in the presence of an interleukin 3 (IL3) in a concentration of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said vector, ~~wherein said concentration range does not cause differentiation of the human pluripotent hematopoietic stem cells.~~

21-22 (Canceled)

23. (Currently amended) A method for obtaining genetically modified human pluripotent hematopoietic stem cells, comprising:

a) contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of human pluripotent hematopoietic stem cells cultured with fibronectin and in the presence of an effective amount of thrombopoietin (TPO), a flt3 ligand (FL), and interleukin-6 (IL-6), wherein the

TPO, FL and IL-6 are each provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL and wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors, ~~and~~ wherein said human pluripotent hematopoietic stem cells are CD34⁺Thy-1⁺Lin⁻ cells and can differentiate into any hematopoietic cell type, and wherein said concentration range does not cause differentiation of the human pluripotent hematopoietic stem cells; and

b) obtaining said modified human pluripotent hematopoietic stem cells.

24. (Previously presented) The method of claim 23, further comprising culturing the human pluripotent hematopoietic stem cells in the presence of leukemia inhibitory factor (LIF) in a concentration range of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said vector.

25. (Previously presented) The method of claim 23, further comprising culturing the human pluripotent hematopoietic stem cells in the presence of interleukin-3 (IL-3) in a concentration range of about 5 ng/mL to about 100 ng/mL prior to contacting said cells with said vector.

26. (Previously presented) The method of claim 23, further comprising culturing the human pluripotent hematopoietic stem cells in the presence of a c-kit ligand in a concentration range of about 5 ng/mL to about 100 ng/mL prior to contacting said cells with said vector.

27-30. (Canceled)

31. (Previously presented) The method according to claim 23, wherein the effective amount of TPO and FL individually is in the range of about 5 ng/mL to about 200 ng/mL and the effective amount of IL-6 is in the range of about 10 ng/mL to about 100 ng/mL.

32. (Previously presented) The method according to claim 23, wherein the vector is a retroviral vector.

33. (Previously presented) The method according to claim 23, wherein the heterologous gene is a marker gene.

34. (Previously presented) The method according to claim 23, further comprising expanding the modified human pluripotent hematopoietic stem cells.

35-36. (Canceled)

37. (Currently amended) A method of transducing human pluripotent CD34 Thy-1 Lin hematopoietic stem cells, comprising:

a) obtaining a source of said stem cells, wherein said stem cells can differentiate into any hematopoietic cell type;

b) culturing said cells with fibronectin and the cytokine thrombopoietin (TPO), flt3 ligand (FL), and interleukin-6 (IL-6), individually provided in the range of about 0.1 ng/mL to about 500 ng/mL, wherein said concentration ranges do not cause differentiation of the human pluripotent hematopoietic stem cells;

c) infecting the cultured cells with a retroviral vector including a polynucleotide sequence encoding a heterologous gene; and

d) obtaining transduced cells wherein said gene is expressed.

38. (Previously presented) The method according to claim 37, wherein the TPO, FL and IL-6 are individually provided in the range of about 5 ng/mL to about 200 ng/mL.

39. (Previously presented) The method according to claim 37, further comprising culturing the cells in the presence of leukemia inhibitory factor (LIF) in a concentration range of about 5 ng/mL to about 200 ng/mL.

40. (Currently amended) The method according to claim 37, further comprising culturing the cells in the presence of IL-3 in a concentration of about 10 ng/mL to about 100 ng/mL, wherein said concentration range does not cause differentiation of the human pluripotent hematopoietic stem cells.

41. (Previously presented) The method according to claim 39, further comprising culturing the cells in the presence of IL-3 in a concentration range of about 10 ng/mL to about 100 ng/mL.

42. (Previously presented) The method according to claim 37, wherein said IL-6 is in the range of about 10ng/mL to about 100 ng/mL.

43. (Previously presented) The method according to claim 37, wherein the TPO is provided as a mimetic.

44-45 (Canceled)

46. (Previously presented) The method according to claim 37, wherein the heterologous gene is a marker gene.

47. (Previously presented) The method according to claim 37, wherein the heterologous gene is a therapeutic gene.

48-51 (Canceled)

52. (Currently amended) A method for obtaining genetically modified human pluipotent hematopoietic stem cells, comprising:

a) contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of human pluripotent hematopoietic stem cells cultured with fibronectin and in the presence of an effective amount of a mpl ligand and a flt3 ligand, each ligand provided in a concentration range of about 0.1 ng/mL to about 500ng/mL, and optionally in the presence of one or more cytokines selected from: c-kit ligand in a concentration range of about 5 ng/mL to about 200ng/mL, interleukin 3 (IL-3) in a concentration range of about 5ng/mL to about 200ng/mL, leukemia inhibitory factor (LIF) in a concentration range of about 5 ng/mL to about 200 ng/mL, wherein said vector is selected from a group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors, and wherein said stem cell can differentiate into any hematopoietic cell type, and wherein said concentration ranges do not cause differentiation of the human pluripotent hematopoietic stem cells; and

b) obtaining said modified human pluipotent hematopoietic stem cells.

53. (New) A method of promoting the expansion of a population of human pluripotent hematopoietic stem cells comprising culturing *in vitro* a human pluripotent hematopoietic stem cell population in a medium comprising fibronectin, and an effective amount of a mpl ligand and a flt3 ligand, wherein each ligand is provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL, wherein said human pluripotent hematopoietic stem cells are CD34⁺Thy-1⁺Lin⁻ cells and can differentiate into any hematopoietic cell type, and wherein said concentration range does not cause differentiation of the human pluripotent hematopoietic stem cells.